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Negative regulation of parathyroid hormone-related protein expression by steroid hormones

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ABSTRACT

Elevated parathyroid hormone-related protein (PTHrP) is responsible for humoral hypercalcemia of malignancy (HHM), which is of clinical significance in treatment of terminal patients with malignancies. Steroid hormones were known to cause suppression of PTHrP expression. However, detailed studies linking multiple steroid hormones to PTHrP expression are lacking. Here we studied PTHrP expression in response to steroid hormones in four cell lines with excessive PTHrP production. Our study established that steroid hormones negatively regulate PTHrP expression. Vitamin D receptor, estrogen receptor α, glucocorticoid receptor, and progesterone receptor, were required for repression of PTHrP expression by the cognate ligands. A notable exception was the androgen receptor, which was dispensable for suppression of PTHrP expression in androgen-treated cells. We propose a pathway(s) involving nuclear receptors to suppress PTHrP expression.

1. Introduction

Hypercalcemia associated with malignancy complicates treatment of advanced cancers, with renal failure, gastrointestinal symptoms, and coma. It is classified into two categories; humoral hypercalcemia of malignancy (HHM) and local osteolytic hypercalcemia (LOH), HHM being more frequent than LOH. Most HHM is due to ectopic production of parathyroid hormone-related protein (PTHrP) by cancer tissues and its action toward bone and kidney [1,2]. Furthermore, PTHrP production by cancer cells metastasized to bone stimulates bone resorption, leading to release of bone-derived TGF-β, which enhances PTHrP secretion, aggravating clinical consequences [3].

Despite efforts made to clarify molecular mechanism of PTHrP production in cancers for the past two decades, molecular mechanism of PTHrP overexpression in malignant cells has been elusive. Noticeably, there are some studies linking signaling molecules to elevated PTHrP expression in cancers [4–7]. However, one signaling pathway toward PTHrP overexpression is dominant in one type of cell line, whereas that pathway does not play a role in elevated PTHrP expression in other cells. For instance, Gli2 expression is correlated with PTHrP secretion in MDA-MB-231 cells, whereas Gli2 mRNA is not detected in another PTHrP-producing cell line, MCF-7 cells. Therefore, forming a coherent picture of mechanism of PTHrP overexpression in cancer cells is still a challenging task.

We have been trying to understand the molecular mechanism of increased PTHrP expression in malignancy. We and others have found that the expression of the PTHrP gene is repressed by1,25-dihydroxyvitamin D3 [8–14]. Here we used four cell lines with elevated PTHrP expression to examine whether steroid hormones, including vitamin D3, estrogen, androgen, glucocorticoid, and progesterone suppressed PTHrP expression. In almost all cases, expression of the nuclear receptors (NRs) appeared to confer an ability to suppress PTHrP expression in response to the corresponding hormone ligands. Depletion of the receptors for vitamin D3, estrogen, androgen, glucocorticoid, and progesterone caused abrogation of PTHrP downregulation upon treatment with the corresponding steroid ligands. On the other hand, the androgen receptor is dispensable for the ligand-induced repression of PTHrP expression. These results suggest existence of a repressive protein complex capable of interacting with liganded NRs.

Abbreviations: PTHrP, parathyroid hormone-related protein; HHM, humoral hypercalcemia of malignancy; LOH, local osteolytic hypercalcemia; TGF, transforming growth factor; NR, nuclear receptor; D3, 1,25-dihydroxyvitamin D3; E2, 17-β-estradiol; DHT, dihydrotestosterone; Dex, dexamethasone; VDR, vitamin D3 receptor; ER, estrogen receptor; AR, androgen receptor; GR, glucocorticoid receptor; PR, progesterone receptor; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; siRNA, small interference RNA; atRA, all-trans retinoic acid.

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2. Materials and methods

2.1. Oligonucleotides and plasmids

Synthetic oligonucleotides for PCR were purchased from Greiner Japan (Tokyo) (Supplementary Table S1). The primer sets for the PTHrP gene detected all the transcripts from the different promoters [5].

2.2. Cell culture and chemicals

HaCaT was a generous gift from Dr. Norbert E. Fusenig and MCF-7, MDA-MB-231, and T47D were provided from Dr. S. Takahashi (the Cancer Institute Hospital, Japan). 1,25-dihydroxyvitamin D3 (D3) is a kind gift from Chugai Pharmaceuticals. Following chemicals were obtained commercially: 17-β-estradiol (E2), dihydrotestosterone (DHT), dexamethasone (Dex), and cycloheximide (Sigma–Aldrich); ICI 182,780 (Tocris Bioscence); all-trans retinoic acid (Wako); and R5020 (Perkin–Elmer). Ligand concentration was 10^{-7} M, except for Fig. S1. All-trans retinoic acid was dissolved in DMSO, whereas the other chemicals were in Ethanol.

2.3. Reverse-transcription and real-time quantitative PCR

Total RNA was isolated with Trizol (Invitrogen) or RNA iso-Plus (TaKaRa Biotechnology, Japan). Total RNA (0.5 μg) was reverse transcribed using random hexamers, oligo(dT) primer, and Prime-Script RT regent kit (TaKaRa Biotechnology). Quantitative real-time PCR analysis was performed by use of the Thermal Cycler Dice TM TP860 (TaKaRa Biotechnology) and SYBR Premix EXTaqII (TaKaRa Biotechnology). Relative gene expression was determined by the δ*CT* method. The data were normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) expression. Multiple preparations of the RNA were subjected to multiple qPCR experiments.

2.4. siRNA

siRNA knock-down was done as reported previously [15]: GR siRNA oligos were from Santa Cruz Biotechnology, siRNA oligos for VDR, ERα, AR and PR were from Dharmacon, and siRNA control was from Cell signaling.

2.5. Statistical analysis

The data were expressed as the mean and the S.D. from three to six independent experiments. Statistical analyses of the mRNA expression levels were conducted using a one-factor ANOVA and Tukey–Kramer test. The differences were considered significant at $P < 0.05$.

3. Results

3.1. Expression of NRs in PTHrP-producing cell lines

Independent studies suggest that steroid hormones, including vitamin D [8–12], dexamethasone [10,16–22] , androgen [23], estrogen [24], and progesterone [25,26] down-regulate expression of PTHrP in several cell types. However, a molecular nature of the

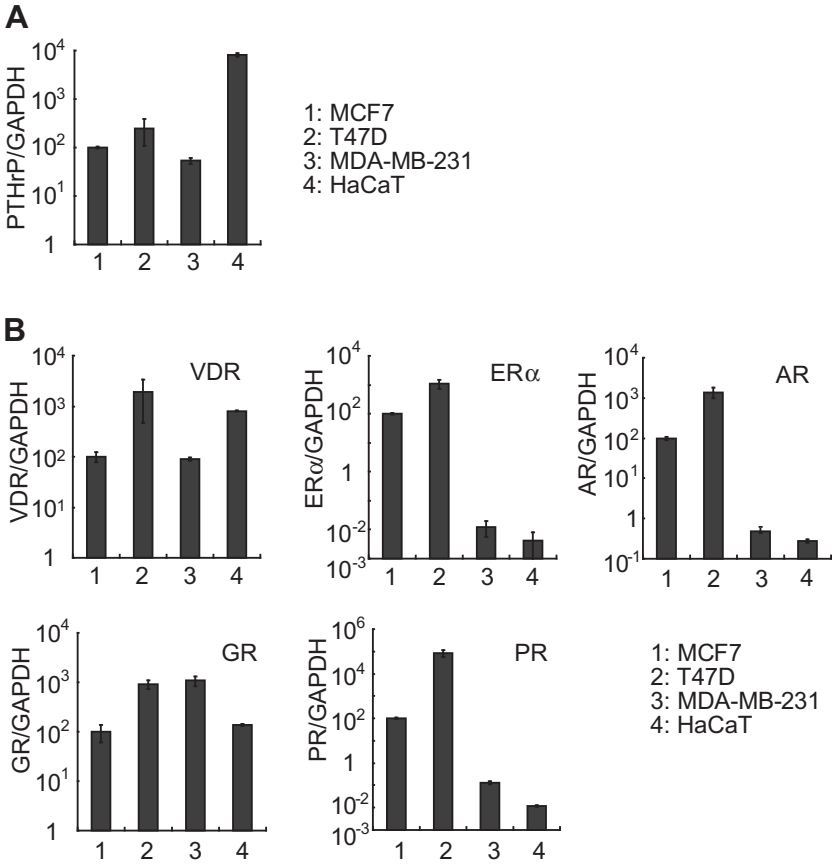

Fig. 1. Expression of PTHrP and NRs in the cell lines. DNA fragments corresponding to PTHrP (A) and NRs (B) were amplified by PCR starting from cDNA reverse-transcribed from RNA of MCF-7, T47D, MDA-MB-231, and HaCaT cells. A ratio of each PTHrP mRNA to GAPDH mRNA in the MCF-7 cells was arbitrarily set to 100 in (A). A ratio of the NR mRNA to GAPDH mRNA in the MCF-7 cells was arbitrarily set to 100 in (B). Each value is represented as a mean ± SD. Lanes 1: MCF-7, lane 2: T47D, lane 3: MDA-MB-231, lane 4: HaCaT.